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Effects of brassinosteroids on quality attributes and ethylene synthesis in postharvest tomato fruit

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ABSTRACT

Effects of brassinosteroids (BRs) on postharvest ripening of tomato fruit were studied in this work. Mature green tomato fruit were harvested and treated with brassinolide (BL, the most active brassinosteroid) or brassinazole (BRZ, a brassinosteroid biosynthesis inhibitor). Following treatment, fruit were stored at 20 °C with 85% RH for 20 days. Fruit quality, respiration rate, ethylene production, lycopene content, chlorophyll content and the expression of ethylene and lycopene biosynthesis related genes, including *golden 2-like* (*LeGLK2*), *phytoene synthase 1* (*LePSY1*), *ripening-related ACC synthase 2* (*LeACS2*), *ripening-related ACC synthase 4* (*LeACS4*), *1-aminocyclopropane-1-carboxylate oxidase 1* (*LeACO1*) and *1-aminocyclopropane-1-carboxylate oxidase 4* (*LeACO4*) were measured. The results showed that during fruit ripening, the application of brassinolide was effective in inducing tomato fruit ripening, increasing soluble sugars, ascorbic acid, lycopene contents, respiration rate and ethylene production, but significantly decreasing chlorophyll content compared with the control. Furthermore, the expression of *LeACS2*, *LeACS4*, *LeACO1*, *LeACO4* and *LePSY1* was increased by brassinolide treatment, while the expression of *LeGLK2* was reduced. However, fruit treated with brassinazole showed the opposite effects, where tomato fruit ripening was delayed. These findings suggest that brassinosteroids are involved in the development of fruit quality attributes and ethylene-mediated fruit ripening of tomato.

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1. Introduction

Ethylene is the major hormone that initiates and controls climacteric fruit ripening, and its biosynthesis has been studied extensively in plant tissues (Srivastava and Handa, 2005; Argueso et al., 2007). There are two systems of ethylene production in plants. System-1 represents basal ethylene in unripe fruit and is regulated in an auto-inhibitory manner, whereas system-2 operates during climacteric fruit ripening and flower senescence and is autocatalytic (Barry and Giovannoni, 2007; Yokotani et al., 2009). Studies have shown that the suppression of ethylene production results in a strong ripening inhibition by knocking down 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ACO) and ACC synthase (ACS) (Hamilton et al., 1990; Oeller et al., 1991). Conversely, application of exogenous ethylene to climacteric fruit at the mature stage stimulates system-2 ethylene biosynthesis, which will accelerate ripening (Nakatsuka et al., 1998).

Brassinosteroids (BRs) are plant steroid hormones known mainly for their effects on cell expansion and a wide range of developmental and physiological processes that occur ubiquitously in plants (Aghdam et al., 2012; Wang et al., 2012; Guo et al., 2013). Extensive studies using genetic, molecular and proteomic approaches have identified most of the major brassinosteroids signaling components, which have been assembled into a series of signal transduction cascades (Kim and Wang, 2010). Research over the past two decades has revealed that brassinosteroids are essential for plant development and regulate a range of physiological processes, such as stem elongation, root growth, leaf epinasty, vascular differentiation and reproductive development (Brosa, 1999; Sasse, 2003). The potential of brassinosteroids to regulate fruit ripening has also been investigated. Application of brassinosteroids to tomato fruit pericarp discs elevated levels of lycopene and lowered chlorophyll levels. Fruit ripening induced by brassinosteroids was associated with increasing in ethylene production (Vidya Vardhini and Rao, 2002). Liu et al. (2014) found that brassinosteroid response transcription factor brassinazole resistant 1 (BZR1)

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mutants could enhance carotenoid accumulation in tomato fruit. However, little is known about the relationship between brassinosteroids and ethylene during tomato fruit ripening. Also, the mechanisms involved in regulation of chlorophyll, lycopene and ethylene of postharvest fruit by brassinosteroids have not been elucidated in detail. Therefore, the aim of this study was to investigate the effects of brassinosteroids on postharvest fruit quality attributes and ethylene synthesis in tomato fruit and the possible mechanisms involved.

2. Materials and methods

2.1. Plant material and chemicals

Tomato fruit (*Solanumly copersicum* L. cv. Yuanbao) were harvested at the mature green stage from an orchard in the Huayang district, Sichuan, China, and immediately transported to the laboratory after harvest. 15–20 fruit of uniform size, maturity and free from visual blemishes and diseases were selected and randomly divided into 7 lots for each assay, which were repeated three times.

Brassinolide (BL, the most active brassinosteroid), brassinazole (BRZ, a brassinosteroid biosynthesis inhibitor), ethephon (ET) and 1-methylcyclopropene (1-MCP) were purchased from Sigma.

2.2. Treatments

The first lot tomato fruit were treated for 12 h by immersion in solutions of 3 μmol L^{-1} brassinolide diluted with distilled water and then air-dried and placed at 25 ± 1 °C. The second lot of fruit were treated for 12 h by immersion in solutions of 5 μmol L^{-1} brassinosteroid diluted with distilled water, and then air-dried and placed at 25 ± 1 °C. For ethylene treatment, one lot fruit was incubated in 500 μL L^{-1} ethephon solution in a closed container at room temperature for 12 h, and then air-dried and placed at 25 ± 1 °C. For the 1-MCP treatment, one lot fruit was placed in 20 L containers and exposed to 0.5 μL L^{-1} 1-MCP gas (SmartFresh™, 0.14% a.i., Rohm and Haas, Philadelphia, PA, USA) for 12 h at room temperature. For the ethephon + brassinazole treatment, one lot fruit was incubated in 500 μL L^{-1} ethephon solution in a closed container at room temperature for 12 h then air-dried and immediately treated with 5 μmol L^{-1} brassinazole solutions for another 12 h and then air-dried and placed at 25 ± 1 °C. For the 1-MCP + brassinolide treatment one lot fruit was placed in 20 L containers and exposed to 0.5 μL L^{-1} 1-MCP gas (SmartFresh™, 0.14% a.i., Rohm and Haas, Philadelphia, PA, USA) for 12 h at room temperature then immersed them in 3 μmol L^{-1} brassinolide solution for another 12 h. The control fruit were treated with distilled water and then air-dried and placed at 25 ± 1 °C. After treatment, tomato fruit were placed in boxes and stored at 23 ± 1 °C with approximately 90% RH for 23 days. Three replicates each of 30 fruit were used for each treatment.

2.3. Measurement of fruit quality parameters

Firmness, soluble sugars content, titratable acidity (TA) and ascorbic acid (AsA) content of the fruit were determined.

Fruit firmness of each individual tomato was measured at three points of the equatorial region by using the FT327 fruit pressure tester (Breuzzi Company, Milano, Italy). The probe penetrated the sample with a uniform force to a depth of 10 mm. The three measurements were averaged for each fruit and expressed in kg/cm. Weight loss was determined by weighing fruit at the start of the experiment and at various intervals during storage. Total soluble sugars of tomato fruit peel and pulp was extracted according to Ozaki et al. (2009). Titratable acidity was determined using the method of Ranggana (1977) by measuring the amount of 0.1 N NaOH. AsA contents of the fruit were measured according to the method of Kampfenkel et al. (1995). Each treatment contained three replicates

2.4. Measurement of total chlorophyll and lycopene content

To measure tomato total chlorophyll contents, about 5 g of fruit peel was extracted in 80% acetone and measured according to Lichtenthaler and Wellburn (1983). The absorbance of samples was read at the wavelength of 645 and 663 nm using a spectrophotometer (TU1800 spectrophotometer, P-general Limited Company, Beijing, China). Total chlorophyll content was estimated as mg/g fresh weight (FW).

Lycopene content was analyzed by the method of Marković et al. (2006). Approximately 5 g samples of fresh tomato peel and pulp was carefully weighed into a 200 mL flask wrapped with aluminum foil to keep out light. The samples of fresh tomatoes were homogenized in a blender. A 100 mL mixture of hexane–acetone–ethanol, 2:1:1 (v/v) was added to the flask and agitated continuously for 10 min on a magnetic stirrer plate. After that, 15 mL of water was added followed by another 5 min of agitation. The solution was separated into distinct polar and nonpolar layers. The hexane solution containing lycopene was filtered through 0.2-mm filter paper; and the filtrate was then diluted with a mixture of hexane–acetone–ethanol (2:1:1, v/v). The residue on the filter paper was colorless, indicating rapid and complete extraction of lycopene. Lycopene concentration was estimated by measuring the absorbance of the hexane solution containing lycopene at 472 nm on a spectrophotometer.

2.5. Measurement of respiration rate

Respiratory oxygen consumption was measured using Clark-type electrodes (Hansatech, King's Lynn, UK) as previously described (Xu et al., 2012). Approximately 0.05 g fruit peel were weighted and cut into small pieces, then pretreated with 5 mL deionized water for 15 min in order to eliminate wound-induced respiration. Measurements were done at 25 °C in a final volume of 2 mL phosphate buffer (pH 6.8), and the cuvette was tightly closed to prevent diffusion of oxygen from the air. Inhibitors of the cytochrome pathway (1 mM KCN) and the alternative pathway (0.5 mM nPG) were used. Total respiration (V_t) is defined as O_2 uptake rate by tomato peel without any inhibitor. The capacity of the alternative pathway (V_{alt}) is defined as O_2 uptake rate in the presence of 1 mM KCN. Residual respiration (V_{res}) is defined as O_2 uptake in the presence of both 1 mM KCN and 0.5 mM nPG. Cytochrome pathway capacity (V_{cyt}) was calculated by the formula: $V_{cyt} = V_t - V_{alt} - V_{res}$.

2.6. Ethylene production

For ethylene production, tomato fruit were placed in a 10 cm × 10 cm closed container for 2 h at 25 ± 1 °C and 85% RH. Then, a 1 mL sample of gas from each container headspace was injected into a FID gas chromatograph (Agilent 6890 Series GC System, Salem, MA) equipped with an activated alumina SS column. The carrier gas (helium) flow rate was 0.5 mL/s. The detector and injector were operated at 100 °C, and the oven was at 50 °C. The ethylene production is expressed as mL/kg/h.

2.7. RNA extraction and qRT-PCR for gene expression analysis

Total RNA was extracted from tomato fruit according to Xu et al. (2012). RNA contents were calculated by measuring the absorbance value taken at 260 nm. First-strand cDNA was reverse transcribed from DNase I-treated RNA with oligo (dT) as the primer. All gene

expression levels were measured by qRT-PCR. The cDNA was amplified by using SYBR Premix Ex Taq (TaKaRa Bio Inc., Dalian, China). The amplification of the target genes was monitored every cycle by SYBR-green I fluorescence. The C_t (threshold cycle), defined as the PCR cycle at which a statistically significant increase of reporter fluorescence firstly detected, was used as a measure for the starting copy numbers of the target gene. Relative quantitation of gene expression was performed using the comparative C_t method. Three technical replicates were performed for each experiment. *ACTIN1* was used as internal controls. All the qRT-PCR primers are listed in Supplementary Table S1. All the mRNA data were expressed as percent of the corresponding *ACTIN1* transcript levels.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.09.016.

2.8. Statistical analysis

All data are reported as mean ± standard error of the mean for three replicates. Student's *t*-test was used for comparison between different treatments. A difference was considered to be statistically significant when $P < 0.05$.

3. Result

3.1. Changes of brassinosteroid synthesis gene expression during tomato fruit development

According to recent reports (Jia et al., 2011; Chai et al., 2013), we define six ripening stages of "Yuanbao" tomato fruit, including small green (SG), big green (BG), mature green (MG), breaking stage (B), pink stage (P) and red stage (R) respectively at 7, 14, 24, 29, 32, 35 days after anthesis (Fig. 1C). The transcript levels of brassinosteroids biosynthetic genes *LeDET2* were increased in SG fruit, but decreased rapidly up to the B stage, then remain stable from B to R stages (Fig. 1C). This might suggest that feedback regulation mechanisms of brassinosteroid signaling are functional during tomato ripening. This result also suggested that brassinosteroids might play a role in tomato fruit ripening.

3.2. Effects of brassinosteroids on fruit ripening and color development

The ripening of tomato fruit could be accelerated by at least 2 days by brassinolide treatment, while brassinazole treatment could delay fruit ripening when compared with the control fruit (Fig. 1A). Brassinosteroid related gene expression was also altered (Fig. S1). Control fruit remained green over the first 5 days, but brassinolide-treated fruit had obvious color change after 3 days. Moreover, on the ninth day, brassinazole-treated fruit just started to become red, while the control and brassinolide-treated tomatoes already had began to deteriorate, especially brassinolide-treated tomatoes.

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.09.016.

There were significant differences in chlorophyll and lycopene contents between control and treated fruit (Fig. 2A and B). In the brassinolide-treated fruit, the total chlorophyll content decreased sharply throughout the whole ripening process, changing from 35.1 to 6.1 μg/g (Fig. 2A), whereas the lycopene content increased rapidly after 3 days of storage, changing from 8.1 to 72.4 mg/kg (Fig. 2B). However, the brassinazole-treated fruit showed a minor degradation of chlorophyll, which was more than twice that of the brassinolide-treated tomatoes at the ninth day (Fig. 2B). Furthermore, lycopene in brassinazole-treated fruit was significantly lower than in control or brassinolide-treated tomatoes during the whole

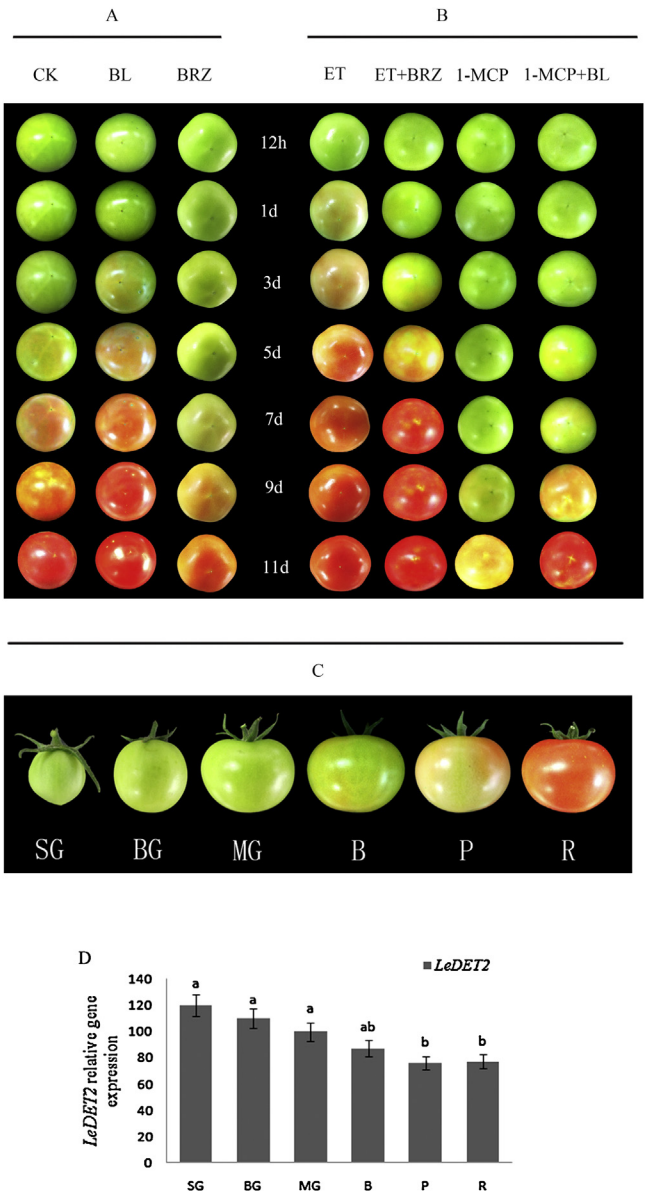

Fig. 1. Effects of different treatments in mature green tomato fruit stored at 25 ± 1 °C. (A) Brassinolide and brassinazole treated tomatoes. (B) Ethephon, 1-MCP, ethephon + brassinazole and 1-MCP + brassinolide treated tomatoes. (C) Quantitative RT-PCR analyses of the transcript levels of BR-synthesis gene *LeDET2* in different tomato development stages. Data followed by different letters are significantly different between samples according to Student's *t*-test at $P < 0.05$. *SG*, small green stage; *BG*, big green stage; *MG*, mature green stage; *B*, breaking stage; *P*, pink stage, *R*, red stage.

ripening process (Fig. 2A). Consistently, the expression of *LePSY1* and *LeGLK2*, which are the key regulators of lycopene biosynthesis and chloroplast development respectively, displayed the same trends as in Fig. 2A and B (Fig. 2C and D).

3.3. Effects of brassinosteroids on fruit quality attributes

Firmness and titratable acidity of tomato fruit decreased gradually with storage time. Brassinazole treatment could delay the decrease of firmness (Fig. 3A) and titratable acidity (Fig. 3B), while brassinolide treatment stimulated these decreases. Soluble sugars content in all fruit showed an increasing trend with storage time, while compared with the control, soluble sugars content was

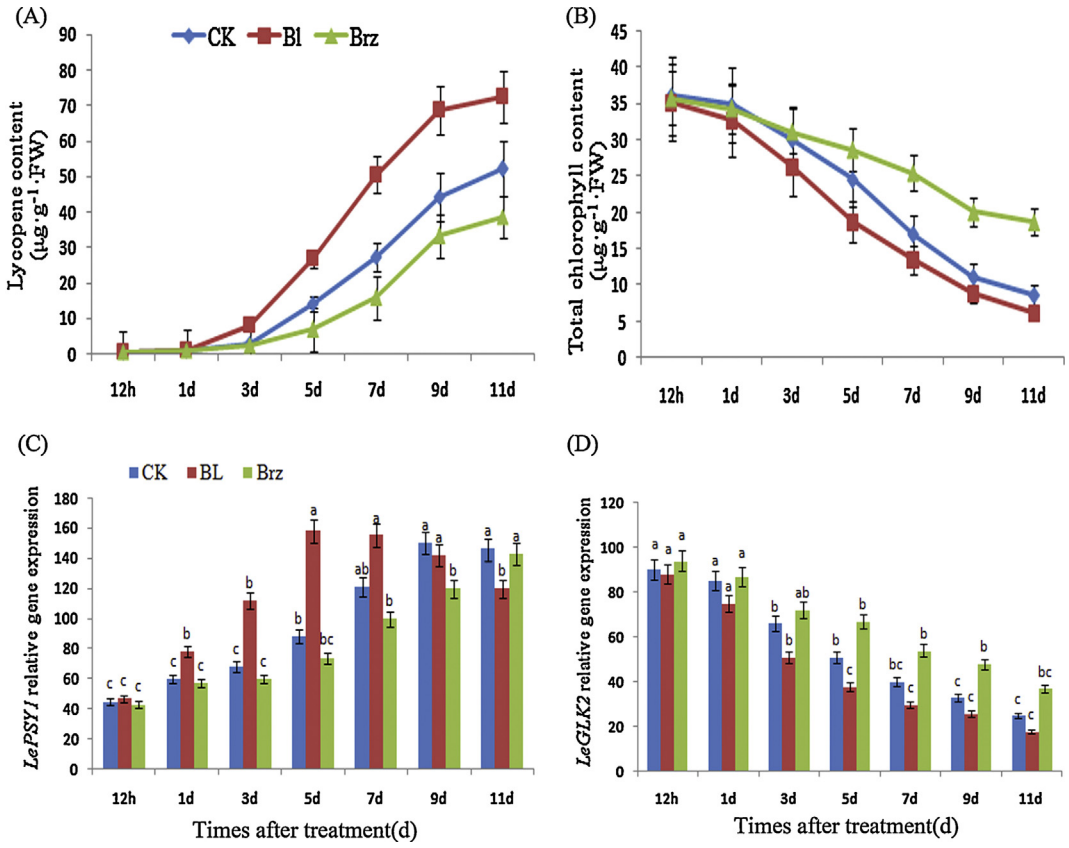

Fig. 2. Effects of brassinolide and brassinazole on lycopene content (A) and chlorophyll content (B) in tomato fruit after treatments. Quantitative RT-PCR analyses of the transcript levels of *LePSY1* (C) and *LeGLK2* (D) during ripening with/without brassinolide or brassinazole treatments. Data followed by different letters are significantly different between samples according to Student's *t*-test at $P < 0.05$.

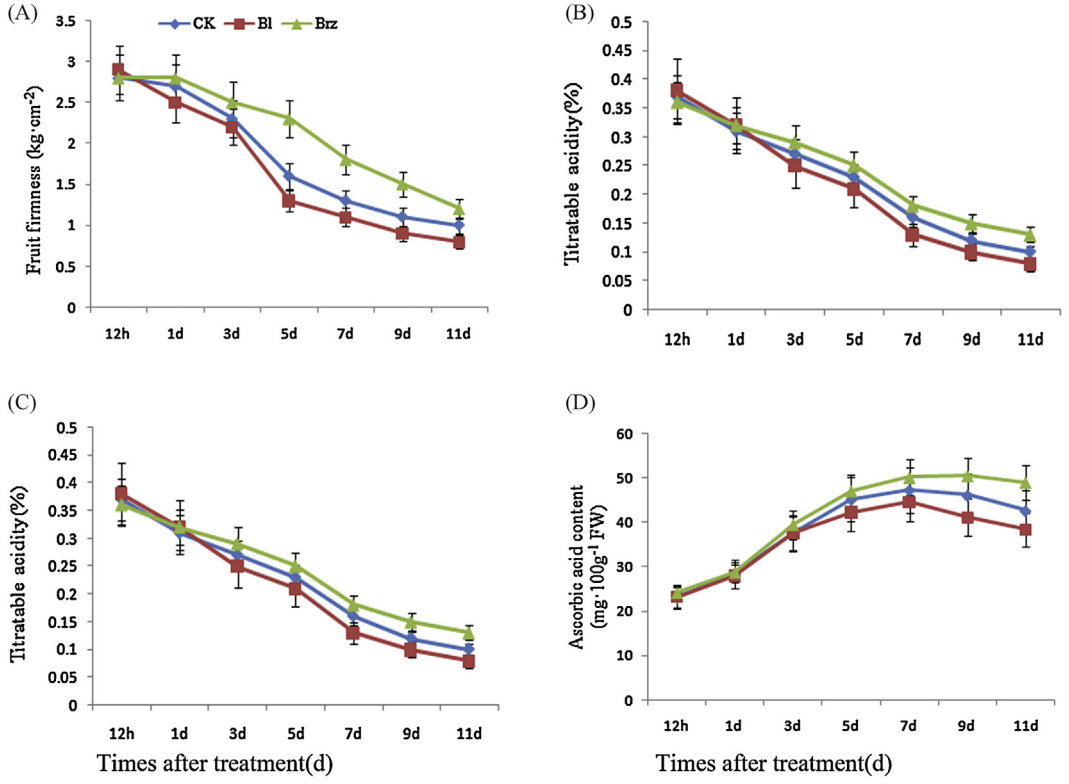

Fig. 3. Effects of brassinolide and brassinazole treatment on fruit firmness (A), titratable acid (B), soluble sugars content (C) and ascorbic acid content (D).

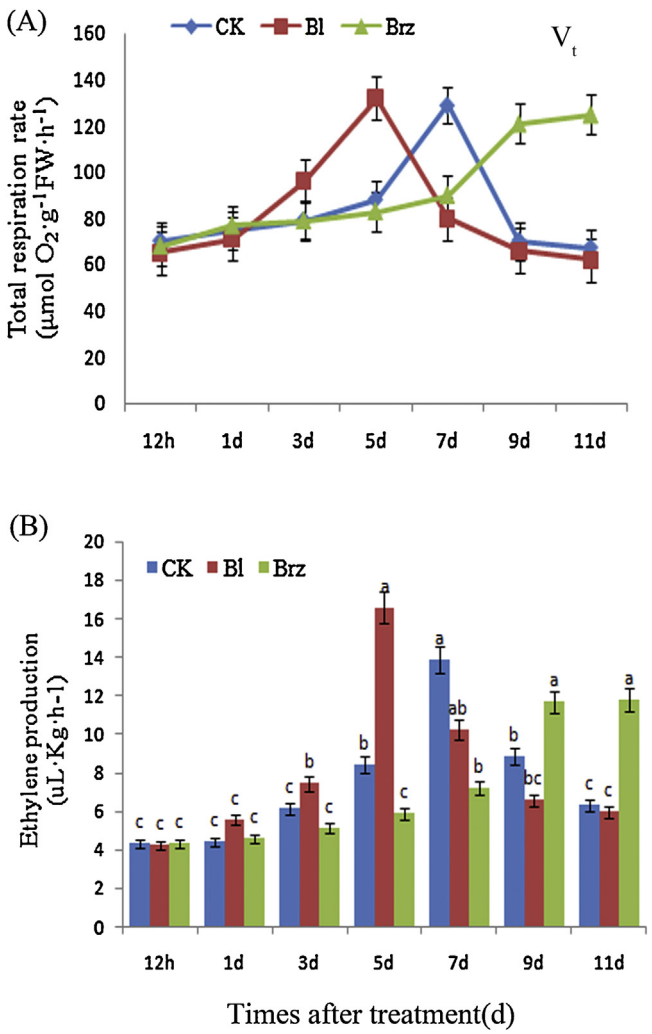

Fig. 4. Changes in the respiration and ethylene production during ripening in control and treated fruit. Total respiration (A), ethylene production (B) were measured after treatment. Data followed by different letters are significantly different between samples according to Student's *t*-test at $P < 0.05$.

higher in brassinolide-treated but lower in brassinazole-treated fruit (Fig. 3C). Ascorbic acid (AsA) content in all fruit increased in the early storage period, and then decreased significantly. Brassinolid-treated fruit had higher AsA contents compared with either control fruit or brassinazole-treated fruit (Fig. 3D). These results indicate that brassinolide treatment had beneficial effects on maintaining quality of tomato fruit in storage.

3.4. Effects of brassinosteroids on respiration rate

Following 7 days of storage, in control fruit, the total respiration rate (V_t) of control fruit reached the respiratory climacteric peak, concomitant with a burst in the cyanide-resistant respiration rate (V_{alt}) (Fig. S2A). Total respiration rate was increased over two fold compared with the initial value in the control fruit (Fig. 4A). Moreover, the cytochrome pathway respiration (V_{cyt}) and cyanide-resistant respiration (V_{alt}) accounts for about 50% and 40% of the total respiration respectively for all the cases (Fig. S2). The total respiratory climacteric peak arrived earlier with the brassinolide treatment than in the control, as well as the cytochrome pathway respiratory and alternative respiratory climacteric peaks. The brassinazole treatment delayed the timing of the total, cytochrome pathway and alternative respiratory climacteric peaks.

Supplementary Fig. S2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.09.016.

3.5. Effects of brassinosteroids on ethylene production of tomato fruit

In our study, a typical climacteric pattern of ethylene production was shown in control fruit, which reached a peak of 13.9 μL/kg/h at the fifth day, and then decreased. It showed a similar trend with the change in respiration rate. Ethylene production was significantly increased after the onset of ripening by brassinolide treatment, and reached the climacteric peak at the fifth days, then gradually decreased after 7 days. Ethylene production with the brassinazole treatment was significantly lower than that in the control fruit during storage (Fig. 4B).

3.6. Effects of brassinosteroids on the expression of LeGLK2 and LePSY1 genes

The expression of *LePSY1* and *LeGLK2* genes in tomato fruit at the mature green stage with/without brassinolide or brassinazole treatment is shown in Fig. 2. *LePSY1* transcript levels in control fruit increased during storage, accompanied with decreased transcription of *LeGLK2*. The expression level of *LePSY1* in brassinolide-treated fruit was significantly higher than in the control, while the expression level of *LePSY1* in fruit treated with brassinazole was lower than in the control (Fig. 2C).

The expression of *LeGLK2* in fruit for all treatments decreased gradually during ripening. However brassinolide treatment accelerated this decrease, and compared with the control, there was only a slight decrease in the expression of *LeGLK2* with the brassinazole treatment (Fig. 2D).

3.7. Effects of brassinosteroids on the expression of ethylene biosynthetic genes

The expression of *LeACS2* and *LeACS4* in control fruit increased sharply and reached a maximum at 7 day and 5 day respectively, and then decreased gradually (Fig. 5). In brassinolide-treated fruit, the expression of *LeACS2* and *LeACS4* showed the same trend as in the control, and transcript levels of these two genes were significantly higher than those in control fruit. In brassinazole-treated fruit, the expression of *LeACS2* and *LeACS4* was depressed significantly when compared with control or brassinolide-treated fruit (Fig. 5A and B).

The expression of *LeACO1* was increased by brassinolide treatment but decreased by brassinazole treatment when compared with control fruit (Fig. 5C). The expression of *LeACO4* showed similar trends with either brassinolide or brassinazole treatments when compared with control fruit (Fig. 5D).

To confirm these results, we treated tomato fruit with 1-MCP or ethephon alone and 1-MCP + brassinolide or ethephon + brassinazole. Ethylene synthesis gene expression is shown in Fig. 6. The expression of *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* was decreased after 1-MCP treatment and increased after ethephon treatment during fruit ripening when compared to control fruit. In fruit treated with 1-MCP + brassinolide, expression levels of these genes were higher than in 1-MCP treated fruit, and still lower than in controls. After ethephon + brassinazole treatment the expression of these genes was lower compared to the ethephon-treated fruit and higher than in control fruit.

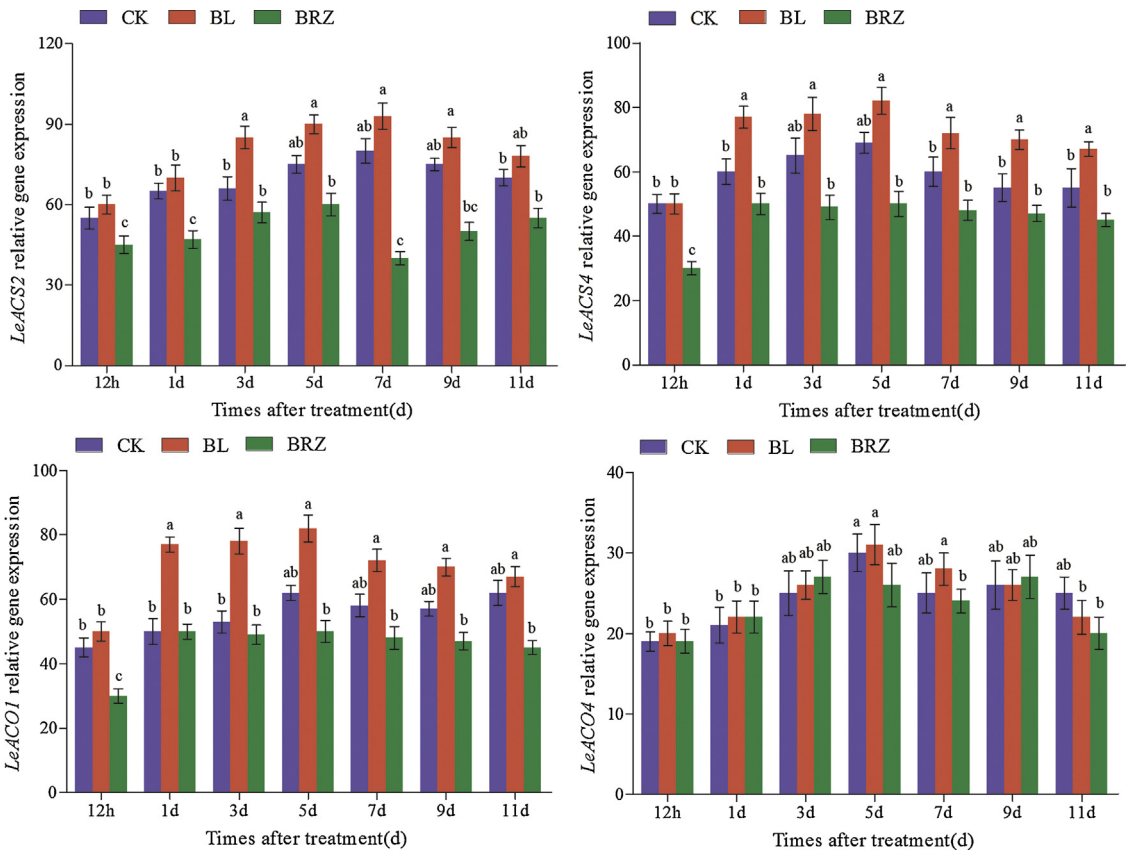

Fig. 5. Quantitative RT-PCR analyses of the transcript levels of ethylene synthesis related genes *LeACS2* (A), *LeACS4* (B), *LeACO1* (C) and *LeACO4* (D) in tomato fruit during ripening with/without brassinolide or brassinazole treatments. Data followed by different letters are significantly different between samples according to Student's *t*-test at $P < 0.05$.

4. Discussion

Fruit ripening is a normal physiological process that reduces quality attributes and limits postharvest storage. Previous studies showed that tomato was a typical climacteric fruit and a good model system for studying the role of ethylene in ripening (Alexander and Grierson, 2002; Barry and Giovannoni, 2007). Postharvest application of brassinolide in fruit has increased along with researc3h on brassinosteroid signaling and functions. In comparison with development and stress resistance, very little is known about the role of brassinosteroids in regulating the biosynthesis of ethylene, as well as nutrient quality during the process of fruit ripening. The use of the specific brassinosteroid biosynthesis inhibitor, brassinazole, is an effective way to clarify brassinosteroid functions (Asami et al., 2001). In this study, we showed that brassinosteroids can accelerate postharvest ripening, enhance development of quality attributes and promote ethylene production in tomato fruit. Our results were consistent with previous reports that brassinosteroids could accelerate ripening of tomato pericarp discs (Vidya Vardhini and Rao, 2002) and grape (Symons et al., 2006). In tomato fruit, the two key enzymes, ACS and ACO, play important roles in regulating ethylene production during ripening. Previous research has shown in tomato fruit that there are at least nine *LeACS* and five *LeACO* subfamilies involved in the regulation of ACS and ACO protein synthesis while *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* are particularly responsible for the massive ethylene production (Barry et al., 1996, 2000; Klee and Giovannoni, 2011). Thus we measured the expression levels of these four genes by different treatments. These results indicated that increased ethylene production in brassinolide-treated fruit was associated with higher transcription levels of *LeACS2* and *LeACS4*.

Lycopene is produced by all photosynthetic organisms as well as by some nonphotosynthetic bacteria and fungi, and synthesized in both dark- and light-grown tissues, such as endosperm, roots, leaves, flowers and fruit via the general isoprenoid biosynthetic pathway in chloroplasts (Ronen et al., 2000; Welsch et al., 2008; Shumskaya et al., 2012). Brassinosteroids have previously been shown to regulate lycopene accumulation (Vidya Vardhini and Rao, 2002). However, the mechanism of how brassinosteroids regulate lycopene biosynthesis has not been investigated. We used qRT-PCR to measure the transcript levels of genes related to lycopene synthesis and chlorophyll synthesis. Our results indicated that brassinolide treatment promoted lycopene accumulation by regulating the expression of the lycopene biosynthetic gene *LePSY1*. *LeGLK2* is a transcription factor that is essential in promoting chloroplast development in tomato fruit (Fitter et al., 2002; Waters et al., 2009; Powell et al., 2012). Brassinolide treatment reduced the transcription level of *LeGLK2*, which leads to a decrease in chlorophyll content during storage compared with control fruit (Powell et al., 2012). These results further suggest that the effects of brassinosteroids in reducing chlorophyll production are correlated with down-regulation of *LeGLK2* gene expression. These effects also might be due to the promotion of ethylene synthesis to some extent, which contributes to expression changes of *LePSY1* and *LeGLK2*. But according to our investigation, the expression levels of *LePSY1* and *LeGLK2* changed immediately after brassinolide or brassinazole treatment, consistent with the expression levels of ethylene synthesis genes. Related to these, application of brassinolide significantly promoted lycopene synthesis but suppressed chlorophyll synthesis via regulating transcript levels of *LePSY1* and *LeGLK2*.

Ethylene plays a critical role in fruit ripening and promotes the transcription and translation of ripening-related genes. In this

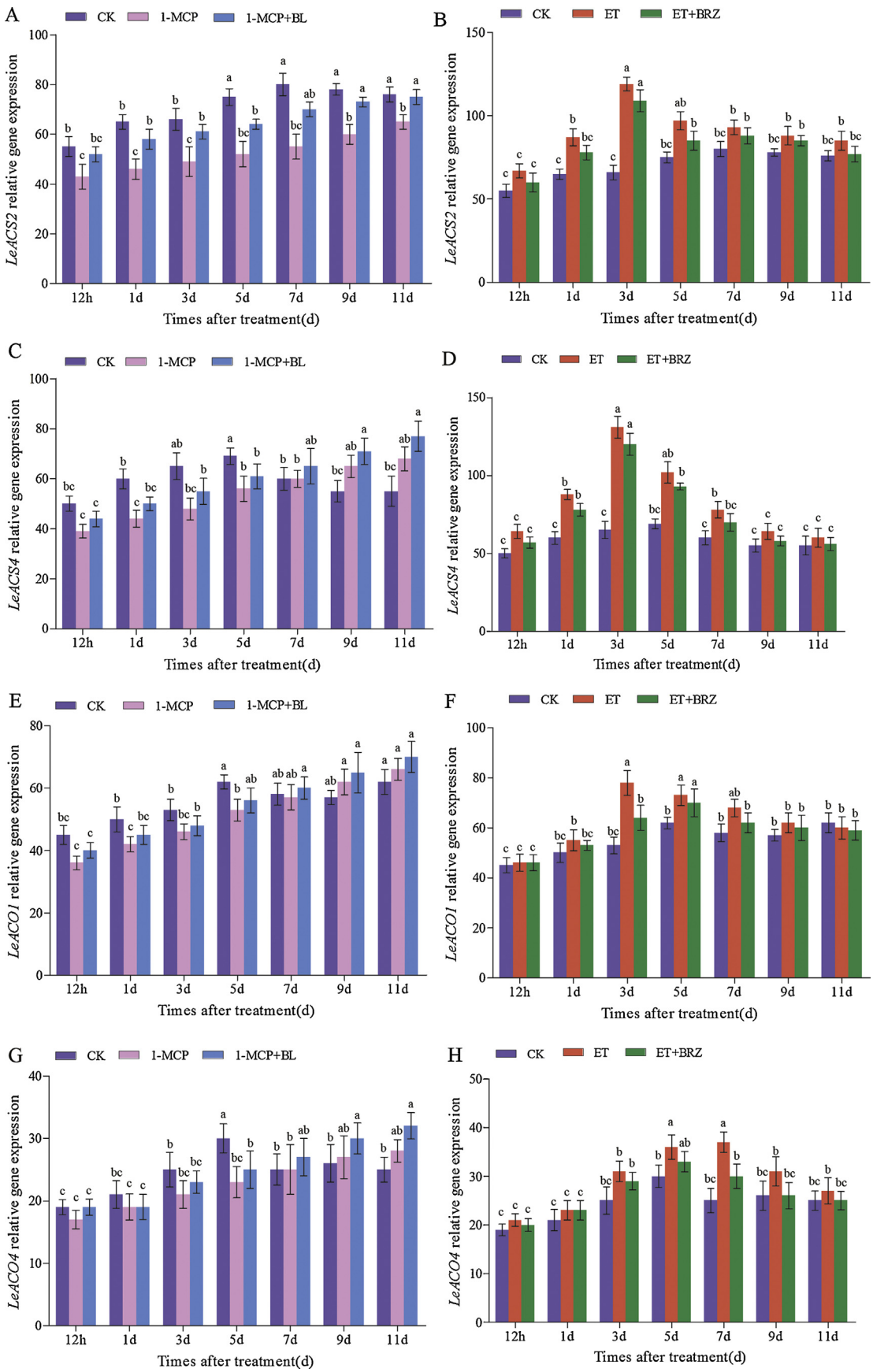

Fig. 6. Quantitative RT-PCR analyses of the transcript levels of ethylene synthesis related genes *LeACS2* (A and B), *LeACS4* (C and D), *LeACO1* (E and F) and *LeACO4* (G and H) in tomato fruit during ripening after ethephon, ethephon + brassinazole, 1-MCP and 1-MCP + brassinolide treatment. Data followed by different letters are significantly different between samples according to Student's *t*-test at $P < 0.05$.

study, ethylene production rate was significantly increased by brassinolide treatment in mature green tomato fruit, while reduced by brassinazole treatment when compared with control fruit. ACS and ACO, two key enzymes, regulated ethylene production during ripening. In particular, *LeACS2*, *LeACS4*, *LeACO1*, and *LeACO4*, members of the ACS and ACO families, are responsible for the massive ethylene production during fruit ripening in tomatoes (Barry et al., 2000; Klee and Giovannoni, 2011; Mazorra et al., 2013). In our study, expression analyses of ethylene synthesis genes such as *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* revealed ACS and ACO transcript levels were significantly increased in brassinolide-treated fruit while reduced in brassinazole-treated fruit (Fig. 5). To verify the results, we treated fruit with 1-MCP, ethephon, 1-MCP + brassinolide and ethephon + brassinazole. As shown in Fig. 6, the expression of these genes were significantly suppressed by 1-MCP treatment but increased by ethephon treatment when compared with control fruit. In 1-MCP + brassinolide treated fruit, *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* genes show a higher expression level than in 1-MCP treated fruit. In ethephon + brassinazole treated fruit, these genes had lower expression levels than in ethephon-treated fruit. Studies have shown that *LeACS2*, *LeACS4*, *LeACO1 and LeACO4* genes are responsible for the burst of ethylene production during ripening of tomato fruit (Nakatsuka et al., 1998), and transgenic tomatoes carrying antisense *LeACS2* produce less ethylene and fail to ripen, with complete inhibition of the *LeACS2* and *LeACS4* genes during ripening (Hamilton et al., 1990), similar to the results reported by Oeller et al. (1991). The expression of *LeACO1* and *LeACO4* genes increased during storage and this increase was promoted by brassinolide treatment. Associated with these, it is suggested that brassinolide treatment may induce ethylene production via promoting *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* gene expression. *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4* are the main genes expressed in ripening of tomato fruit (Lincoln et al., 1993). It is likely that ACS and ACO might act as activation factors to ethylene production by brassinolide treatment in the mature green tomato fruit.

In conclusion, the present study has demonstrated that postharvest application of brassinolide significantly promoted lycopene synthesis but suppressed chlorophyll synthesis via regulating transcript levels of*LePSY1* and *LeGLK2*. Moreover, ethylene production was obviously increased by brassinolide treatment through inducing the expression of ethylene biosynthesis related genes, including *LeACS2*, *LeACS4*, *LeACO1* and *LeACO4*. This effect of brassinosteroids might be due to the promotion of ethylene synthesis to some extent, which contributed to *LePSY1* and *LeGLK2* changing. According to our investigation, the expression levels of *LePSY1* and *LeGLK2* changed immediately after brassinolide or brassinazole treatment, consistent with the expression levels of ethylene synthesis genes. Related to these, application of brassinolide significantly promoted lycopene synthesis but suppressed chlorophyll synthesis partly via regulating transcript levels of *LePSY1* and *LeGLK2* directly and partly through ethylene synthesis. Our study, therefore, suggests previously unknown mechanisms whereby brassinolide or brassinazole treatments might be an effective way to control tomato fruit ripening, at least partly via controlling fruit quality attributes and ethylene biosynthesis.

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